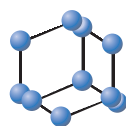


REVIEW ARTICLE

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Advantages of Vasoactive Intestinal Peptide for the Future Treatment of Parkinson's Disease



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Abstract: Parkinson's disease is the second most common neurodegenerative disorder in adults over the age of 65. The characteristic symptoms of Parkinson's disease, such as resting tremor, muscular rigidity, bradykinesia, postural instability and gait imbalance, are thought to be a result of the progressive degeneration of the dopaminergic neurons of the substantia nigra compacta, resulting in insufficient dopamine integrated signalling on GABAergic medium spiny neurons in the striatum. Despite tremendous research, the molecular mechanisms underlying the pathogenesis of neurodegeneration in Parkinson's disease have remained largely unknown. Although a variety of possible pathogenic mechanisms have been proposed over the years, including excessive release of oxygen free radicals, impairment of mitochondrial function, loss of trophic support, abnormal kinase activity, disruption of calcium homeostasis, dysfunction of protein degradation and neuroinflammation, the pathogenesis is still largely uncertain, and there is currently no effective cure for Parkinson's disease. To develop potential therapies for Parkinson's disease, inflammatory processes, mitochondrial dynamics, oxidative stress, production of reactive aldehydes, excitotoxicity and synucleinopathies are to be targeted. In this respect, vasoactive intestinal peptide has beneficial effects that provide an advantage for the treatment of Parkinson's disease. Vasoactive intestinal peptide is a major neuropeptide-neurotransmitter having antioxidant, anti-inflammatory, neurotropic, neuromodulator, and anti-apoptotic properties. In addition to its direct neuroprotective actions regulating the activity of astrocytes, microglia and brain mast cells, it also plays important roles for neuronal adaptation, maintenance and survival.

Keywords: Parkinson's disease, vasoactive intestinal peptide (VIP), 6-hydroxydopamine, oxidative stress, inflammation, Alpha-synuclein.

1. INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder; there is no effective treatment, and it predominantly affects nigral dopaminergic neurons. In addition to motor symptoms (tremor, bradykinesia, rigidity, gait and balance problems, e.g.), Parkinson's symptoms may be nonmotor, such as depression, apathy, sleep disorders, loss of sense of smell and cognitive impairment [1]. The cause/causes of PD are still unknown, but in general, two main factors play a role: genes and environmental risks. Pathophysiological mechanisms that can be realized depending on all these factors are various. Depending on the progressive behaviour of the PD, current therapies can only provide temporary symptomatic relief and slowing, and neuronal loss cannot be restored [2]. The development of effective treatment modalities that can counteract the multifaceted pathophysiology of the disease is still one of the challenges pending today. Vasoactive intestinal peptide (VIP) is a basic endogenous peptide molecule that functions in many physiological processes. Today, it appears to be a molecule that has the potential to counteract different mechanisms of PD molecular pathogenesis, as well as to exhibit positive effects against many different pathologies [3]. This review aims to summarize the direct effects of VIP on PD pathology as a therapeutic agent in the most recent investigations.

2. PARKINSON'S DISEASE

PD is a common neurodegenerative disorder that does not have an effective treatment and is characterized by the degeneration of large-scale dopaminergic neurons in the substantia nigra compacta (SNc) and loss of projecting nerve fibres to the striatum, a major

component of the basal ganglia, the presence of proteinaceous inclusions called Lewy bodies (LBs) and chronic inflammation [1]. The pathogenesis of PD involves several factors, such as α -synuclein aggregation, oxidative stress, mitochondrial dysfunction, and activation of apoptosis, but the exact molecular mechanisms of neurodegeneration remain obscure [4]. The formation of neurotoxic aggregates of the small presynaptic protein α -synuclein is currently thought to be a main event in PD pathogenesis. The aggregation of α -synuclein is presumably induced by several factors, including point mutations and multiplication of the α -synuclein gene, intense oxidative stress, mitochondrial dysfunction, damage to protein degradation systems, etc. [5, 6]. Dysregulation of apoptosis in dopaminergic neurons of the SNc is considered one of the possible mechanisms causing their death [6]. The possible roles of inflammation and autophagy in PD pathogenesis have been discussed [7]. Studying the mechanisms of neurodegeneration in monogenic PD forms with a known molecular cause could provide a better understanding of the pathogenesis in more common sporadic forms. To date, 18 loci of the human genome have been associated with hereditary forms of PD [8]. PD forms with Mendelian inheritance were demonstrated to be due to mutations in at least five genes, namely, the genes for α -synuclein (SNCA), E3 ubiquitin ligase (parkin, PARK2), mitochondrial kinase (PINK1), a redox-dependent chaperone (DJ1), and LRRK2. While PARK2, DJ1, and PINK1 mutations are associated with rare autosomal recessive early-onset PD forms (usually developed before 40 years of age), LRRK2 mutations are found in a more common autosomal dominant late-onset form and in sporadic PD. Thus, LRRK2 mutations are a frequent cause of hereditary PD [9].

There is a "chicken and egg situation" comprising an interactive loop between mitochondrial dysfunction, protein misfolding and immune response alterations. At the molecular level, there is a decrease in mitochondrial function due to the following changes:

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changes in the function of the electron transport chain, a loss of the electrical and chemical transmembrane potential of the inner mitochondrial membrane or a reduction in mitochondrial transport of critical metabolites [10]. All of these changes result in reduced oxidative phosphorylation efficiency and a reduction in adenosine-5'-triphosphate (ATP) production [11]. Degraded mitochondrial function leads to increased oxidative stress and affects various cellular pathways leading to damage to intracellular components and cell death. Oxidative stress is one of the pathogenic mechanisms of nigral dopamine cell death in PD [12].

3. VASOACTIVE INTESTINAL PEPTIDE

VIP is a basic 28-amino acid peptide that was first extracted from pig small intestine [13] and is expressed in numerous metabolically active organs such as the intestines, pancreas, urogenital tract, thyroid, adrenal glands, and the hypothalamus [14-16]. VIP plays a role in numerous biological activities, including vasodilation and smooth muscle relaxation, stimulation of pepsinogen secretion by the chief cells of the gut, and secretion of water and electrolytes into the intestines [14, 17, 18]. VIP has also been shown to play an important role in modulating the immune system [3, 19]. It has been detected in several key organs of the immune system, including the thymus, spleen, and lymph nodes. VIP is released from nerve terminals and immune cells within lymphoid organs and acts as a potent anti-inflammatory factor by regulating the production of both anti- and pro-inflammatory mediators [20].

Although VIP was initially classified as a gut hormone, evidence from many studies over the past 30 years has demonstrated that it also acts as a neurotransmitter in both the peripheral and central nervous system (CNS) [21]. VIP is widely expressed throughout numerous brain regions, with the highest expression levels found in the cerebral cortex, hypothalamus, hippocampus and amygdala [22]. It has been shown that VIP increases cerebral cortex glycogen metabolism [23], regulates embryonic growth [24] and improves neuronal survival [25]. Many studies have demonstrated that the neuroprotective properties of VIP are facilitated by promoting the expression and secretion of astroglia-derived factors in the presence of toxins [26]. Likewise, VIP is also known to play a crucial role in driving mammalian circadian rhythms, which are partly regulated by the hypothalamus-pituitary-adrenal (HPA) axis [27, 28]. VIP knockout mice do not show a daily rise in circulating corticosterone in response to light and show deficiencies in circadian behaviour, for example, poor motor rhythmicity in constant darkness and improper responses to phase-shifting light pulses [28]. Thus, it is apparent that VIP functions as both an important endocrine and neuronal factor in many locations throughout the body.

The effects of VIP are mediated by three different G protein-coupled receptors (GPCRs), VPAC1, VPAC2, and PAC1, that are specific membrane receptors belonging to the superfamily of GPCRs [29]. Although receptors activated by VIP are widely distributed throughout the entire body, VPAC1 and VPAC2 receptors exhibit slightly different expression patterns. VPAC1 is constitutively expressed, while the expression of VPAC2 needs stimulation [26]. VPAC1 receptors are found in many tissues and organs such as the liver, kidney, prostate, breast, spleen, lung, gastrointestinal tract, and lymph nodes. Within the CNS, VPAC1 is expressed in the pyriform cortex, putamen, supraoptic nucleus, choroid plexus, dentate gyrus, and the pineal gland. VPAC2 receptors are predominantly present in smooth muscle layers of organs and blood vessels as well as the cerebral cortex, periventricular nucleus, suprachiasmatic nucleus, thalamus, hypothalamus, and amygdala of the CNS [30]. Nevertheless, anatomical mapping of these receptors demonstrates one or both receptors in regions of the body in which VIP effects are described, such as the gastrointestinal tract and CNS.

Within the realm of drug discovery and pharmacotherapeutics, recent research and clinical findings have focused on the exploitation

of class-II GPCRs in the development of new therapeutic agents. Ligands that activate these receptors, such as VIP, have been shown to be beneficial, and these receptors have been considered drug targets for the treatment of numerous neurological disorders. VIP is a neuropeptide with potent antioxidant, anti-apoptotic and anti-inflammatory properties [31]. These properties, along with the anti-glutamatergic function of VIP, are well documented in various tissues, including neural tissue, and pathological conditions such as PD, septic shock [32], haemorrhagic shock [33], rheumatoid arthritis [34], acute respiratory distress syndrome [35], ischaemic-reperfused retina [36] and brain trauma [37]. VIP can also pass through the blood-brain barrier (BBB) via transmembrane diffusion [38].

4. VIP AND ITS ROLE AGAINST PARKINSON'S DISEASE RELATED ABNORMALITIES

4.1. Mitochondrial Dysfunction And VIP

Mitochondrial dysfunction in dopaminergic neurons of idiopathic and familial PD is well known, although the underlying mechanisms are not clear. Mitochondrial dysfunction is mainly characterized by the formation of reactive oxygen species (ROS), a reduction in mitochondrial complex I enzyme action, cytochrome-c release, ATP reduction and caspase 3 stimulation [39]. Systemic mitochondrial complex I deficiency plays a role in the pathogenesis of idiopathic PD, which may produce additional oxidative stress in nigral neurons [40]. Degraded mitochondrial function leads to increased oxidative stress and accelerates the degeneration of neurons and interferes with the integrity of neurons [40]. The oxidative stress or ROS not only directly activates cellular damage but also activates signalling pathways leading to cell death [11]. The most direct evidence for impaired mitochondrial metabolism has been obtained from *in vitro* cell cultures using autopsy tissues and other tissue samples from human patients with PD [11]. In addition, mitochondrial abnormalities have also been reported in mouse and fruit fly models of PD [41-43]. In conclusion, postmortem studies of the brains of sporadic PD patients have highlighted the presence of oxidative damage in the pathogenesis of PD, as evidenced by damaged/oxidized lipids, proteins and DNA molecules by strong oxidant radicals, especially in the SNc of the brains [44].

Antioxidant effects of VIP have been demonstrated in many different tissues, including renal [32, 45], alveolar epithelium [46], corneal [47], testicular [48], liver [32], pancreatic [49] and neuronal tissue. In a study of Offen and his colleagues on neuronal cultures, remarkably low concentrations of VIP protected against dopamine and 6-hydroxydopamine (6-OHDA) toxicity in PC12 and neuroblastoma cells by the depletion of reduced glutathione (GSH) [50]. Therefore, it has been suggested that a mechanism of neuroprotection by VIP may be mediated through raising cellular resistance against oxidative stress, and this suggestion was also proven by a subsequent work [51]. Glutamate toxicity can deplete neuronal GSH, which hinders the cells' capability to reduce reactive oxygen species, which ultimately culminates in the death of the cells by oxidative stress. Neurons resistant to glutamate toxicity display increased phosphorylation of cAMP-response-element binding (CREB) and decreased ERK1/2 suggestive of differences in signal transmission [52]. It was shown that the amounts of candidate GPCRs involved in this resistance are important, and an increase in mRNA for receptors (VPAC2) activated by VIP was observed [53]. Similarly, VIP may exert protective effects via modulating oxidative stress and apoptosis in manganese-induced neurodegeneration in NE-4C neural stem cells *in vitro* [54]. Thus, VIP seems to be a good candidate for *in vivo* use against oxidative stress resulting from mitochondrial dysfunction, which is one of the possible mechanisms of neurodegeneration observed in Parkinson's disease.

4.2. Inflammation and VIP

Current available data indicate that neuroinflammation and chronic inflammation of the CNS may play a critical role in the development of many neurodegenerative diseases, including PD. In PD, neuroinflammation has been shown to play an important role in the initiation and progression of dopaminergic neuronal loss, which is the most prominent feature of the disease [55]. Excess proliferation and activation of microglial cells as well as increased proinflammatory mediators such as tumour necrosis factor (TNF)- α , interleukin (IL)-6, IL-1 β , nitric oxide (NO) and ROS can be observed in both cerebrospinal fluid and brain tissue of PD patients indicating inflammation [56]. It was suggested that proinflammatory cytokines, particularly TNF- α , may be involved in neuronal cell death. Furthermore, mediators released from dying neurons caused additional activation of microglial cells and produced a vicious cycle, an inflammatory vicious cycle, that resulted in enhanced neuronal cell death by inflammation (Fig. 1).

Innate immune responses serve as the first defence against pathogens and endogenous insults. For this purpose, the innate immune system primarily acts to initiate a nonspecific response to any possible compound and/or potential threat, which is unnecessary. In PD, activation of the adaptive immune system is ensured after various components of the innate immune system are activated and the integrity of the BBB is impaired [58]. Astrocytes constitute a significant portion of the glial cell population in the CNS, and their function is to preserve the integrity of the BBB, facilitate repair and scar formation, and maintain extracellular ion homeostasis [57]. The expression of receptors that are critical for innate immunity, such as Toll-like receptors (TLRs), double-stranded RNA-dependent protein kinase, nucleotide-binding oligomerization domains, mannose-binding lectin receptor, scavenger receptors, and

complement system components, has suggested a role for astrocytes in innate immunity [56]. Astrocytes can produce neurotrophic factors such as nerve growth factor (NGF), neurotrophin-3 (NT-3) and basic fibroblast growth factor (bFGF), as well as source metabolic substrates such as lactate and GSH for neuronal survival and proper functioning. Astrocytes also remove extracellular excitotoxic agents such as glutamate, potassium, and calcium from the media to provide neuroprotection [56]. On the other hand, during gliosis in response to neuronal damage or toxic insults, astrocytes secrete cytokines and chemokines harmful to the neurons along with microglia [59]. Recent findings have also shown that complex molecular interactions between astrocytes and neurons are essential for neuronal protection. Studies demonstrate that neonatal astroglia and postnatal astrocytes possess receptors for VIP [60, 61]. It has been shown that VIP signalling enhanced glutamate uptake by astrocytes, increased astrocytic release of IL-6, and indirectly reduced the toxicity of glutamate. These results demonstrated that VIP is necessary for the molecular interaction of neurons and astrocytes and plays a role in regulating the protective effects of astrocytes for neurons [62]. The role of astrocytes in PD is controversial and not well understood. Studies on whether astrocytes have a neuroprotective effect and/or neurotoxic effect on PD are insufficient. However, astrogliosis has been reported in some cases of PD as astrocytes are activated by an increase in glial fibrillary acid proteins (GFAP) [56]. GFAP is an intermediate filament that astrocytes need to synthesize cytoskeletal structures and is a good biomarker for astrogliosis [63]. In addition, active astrocytes have been reported in postmortem brains of PD patients; however, the situation was not clarified because this activation was not limited to SNc alone [64]. However, a recent study in a 6-OHDA Parkinson rat model demonstrated that 6-OHDA lesions significantly increased the density of astrocytes in the stri-

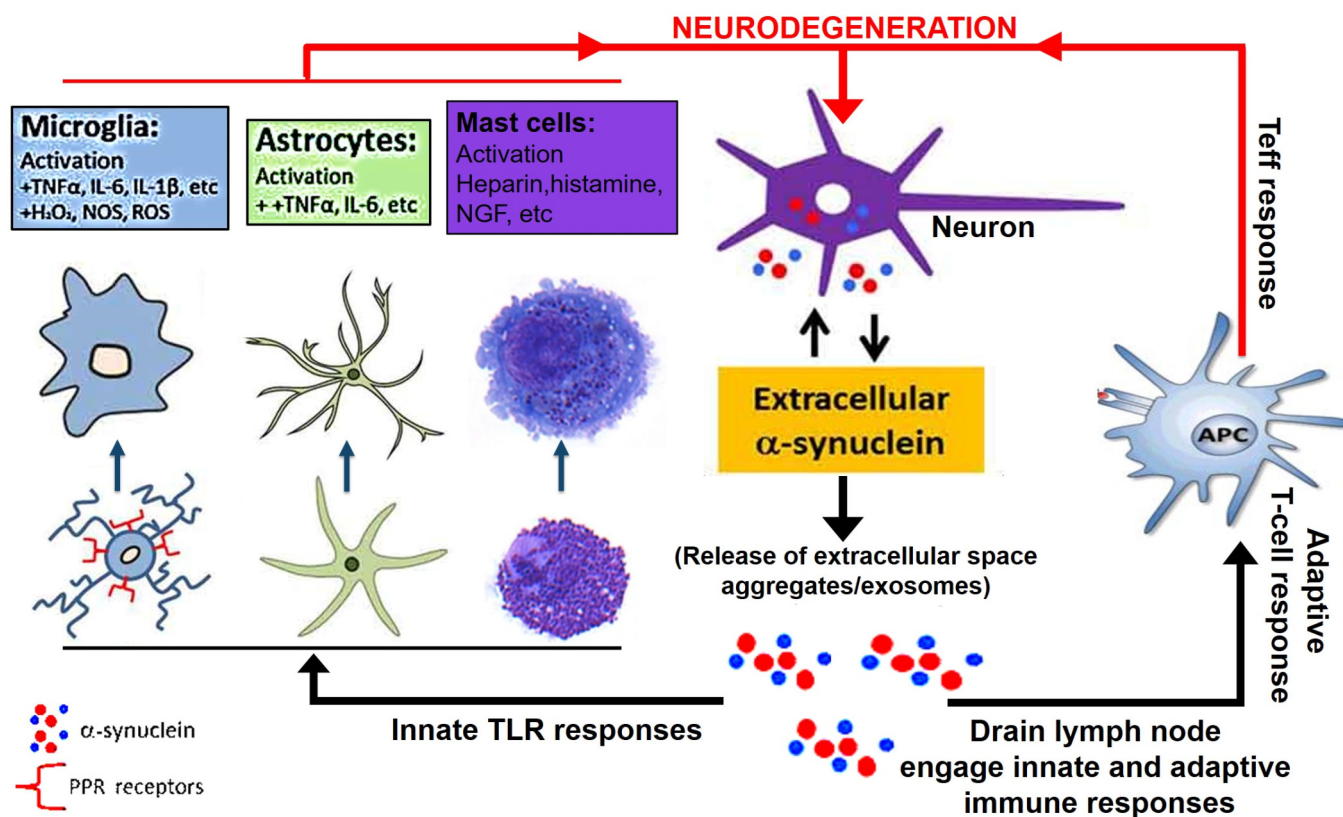


Fig. (1). The mechanism of neurodegeneration due to alpha-synuclein-induced neuroinflammation. The -synuclein (or another molecule) aggregates/exosomes initiate neurodegeneration through innate immune systems with H_2O_2 etc) and mast cells (histamine, serotonin, NGF etc) and/or through the adaptive immune system with Teff-cells (TLR: tool like receptors, PPR: pattern recognition receptor, APC: antigen-presenting cell).

tum, and VIP treatment slightly reduced the gliosis. VIP treatment also reduced GSH levels, and VIP acted primarily on neurons to increase activity-dependent neuroprotective protein (ADNP) and glutamic acid decarboxylase (GAD) expression for protection. The same study suggested that, in the 6-OHDA-induced neurodegeneration model, astrocytes were possibly activated for forefront defensiveness by modulating striatal neurochemistry [65].

Unlike astrocytes, microglial activation and the concomitant inflammatory response have been reported in both PD and animal models. As a resident CNS macrophage, microglia cells are responsible for clearing the CNS environment of potentially leaking pathogens and/or endogenous insults. As a result, their phagocytic, cytotoxic and antigen-presenting abilities provide protection for the CNS from various infiltrations [66]. Activated microglia infect pathogens and damaged cells by releasing toxic ROS and free radicals and through phagocytosis. In PD, the expression of enzymes responsible for producing these toxic species, such as NADPH oxidase (NOX or PHOX), induced nitric oxide synthase (iNOS) and myeloperoxidase (MPO), are increased in the SNc [67]. These reactive species can activate microglial cells, and activated microglial cells release pro-inflammatory cytokines, such as, TNF- α , IL-1 β and IL-6, to attract lymphocytes in the inflammatory process. In addition, these cytokines can indirectly induce more production of ROS and pro-inflammatory cytokines or may directly cause receptor-mediated cytotoxicity [56]. The activated microglia, scavengers, and inflammatory mediators such as cytokines and chemokines, which contribute to the pathophysiological changes associated with various neuroimmunologic disorders, contribute to PD as well. VIP inhibited the expression of the microglia-derived chemokines and inhibited the production and binding of nuclear factor kappa-B (NF- κ B) through the specific receptor VPAC1 [68], and VIP had a clear neuroprotective effect on inflammatory conditions resulting from brain trauma/lipopolysaccharide (LPS)-activated microglia by inhibiting the production of microglia-derived proinflammatory factors such as TNF- α , IL-1 β , IL-6 NO [37, 69]. VIP can also suppress microglial activation through inhibition of the inducible cyclooxygenase-2 (COX-2)/ prostaglandin E2 (PGE2) system [70]. Microglial activation is also an important factor for Alzheimer's disease (AD) pathology. AD is characterized by extracellular deposits of fibrillar β -amyloid in the brain, increased microglial-mediated inflammatory reactions in senile plaques, selective neuronal death, and cognitive deficits. VIP prevented β -amyloid-induced neurodegeneration by indirectly inhibiting the production of a wide range of inflammatory and neurotoxic agents by blocking signalling through the p38 MAPK, p42/p44 MAPK, and NF- κ B cascades that are involved in the transcription of inflammatory mediators and the production of free radicals by β -amyloid-activated microglia cells [71]. VIP also markedly increased microglial phagocytosis of fibrillar β -amyloid and this enhanced phagocytotic activity depended on activation of the protein kinase C (PKC) signalling pathway in the hippocampus of a transgenic mice model of AD [72].

The adaptive immune response is a highly specific, B- and T-lymphocyte-mediated response to harmful agents characterized by humoral and cell-mediated responses, respectively, and these cells are stimulated by precise antigens and directly induce toxicity and cell death to the antigen-expressing cell [73]. The adaptive immune cells need an antigen-presenting cell to initiate B and/or T cells to recognize a specific antigen. In PD, innate immune activation leads to increased BBB permeability, allowing infiltration of peripheral T cells and B cells [56]. These infiltrating cells are activated by the introduction of endocytosis peptides into the relevant cells by active microglia expressing MHC I/II. Evidence that individuals with single nucleotide gene polymorphisms in the MHC class II are predisposed to PD also suggests that both the adaptive immune system and the innate immune system may be involved in PD pathogenesis [74]. Although the adaptive immune system seems to make a de-

layed contribution to the pathology of PD, it is important to understand that therapies can alleviate and counteract the pathology that this system causes.

VIP was a potent mediator of T-cell function by altering developmental responses of thymocytes to T-cell receptor-dependent stimulation and could be a mediator that clarifies the unique thymic microenvironment for topographically precise development of T cells [75]. VIP also inhibited the expression of MHC class II antigens on astrocytes [76, 77]. VIP is produced within the lymphoid microenvironment and modulates several immunological functions. The expression of VIP mRNA in thymocyte subpopulations and T-cell lines indicates that antigen-specific T cells as well as thymocytes may function as VIP sources in lymphoid organs [78]. Although primarily anti-inflammatory in nature, VIP also affected resting macrophages, and macrophage-induced regulation of Th1/Th2 differentiation by inhibition of Th1 development or inducing differentiation of Th2 effector cells and could promote the phenotypic and functional maturation of dendritic cells [79, 80]. In addition, Th2, but not Th1 cells, expressed and secreted functional VIP following specific antigen stimulation [81]. VIP inhibited IL-2 and IL-4 production in thymocytes through different molecular mechanisms [78]. Briefly, VIP release in the lymphoid microenvironment following antigenic stimulation provided a physiological basis for the immunoregulatory effects of VIP on neighbouring immune cells, for example, effects on lymphocyte migration, on T-cell differentiation, and on antigen-induced T-cell apoptosis or downregulation of macrophage activation.

Since the invasion of T cells in the brain was first reported [82, 83], numerous studies have confirmed the infiltration of immune cells in the brain in both postmortem PD patients and animal models [84, 85]. These brain-infiltrating lymphocytes consisted of a heterogeneous population of both CD8 + and CD4 + cells. The presence of a similar profile of peripheral leukocyte infiltration in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse models of PD further confirmed the association of the model with the human syndrome [84]. Effector T (Teff)-cell responses have been shown to exacerbate neuroinflammation and neurodegeneration [85]. The molecular and antigenic bases of these T-cell-mediated neurodegenerative activities have not yet been fully understood. In contrast, regulatory components of adaptive immunity affected neural repair and protection. It has been shown that neuroinflammatory-based neurodegenerative processes can be slowed down by T-cell-mediated regulation [86]. The cellular basis for this neuroprotective response was the CD4+ T-cell population, suggesting a connection to CD4+CD25+ regulatory T cells (Tregs), cells known to suppress immune activation and maintain immune homeostasis and tolerance [87]. While still under investigation, the infiltration of Tregs was generally accepted to have protective effects on dopaminergic neurons through suppressing microgliosis either by direct contact or secreting cytokines that attenuate inflammatory responses [85, 87]. In addition to deactivating macrophages, dendritic cells and microglia, VIP changed the Th1/Th2 balance, causing the proinflammatory Th1 effect to be damaged by promoting preferential differentiation and survival of Th2 cells. Various mechanisms work on the VIP-triggered Th1/Th2 shift. It has been reported that a novel mechanism for the effect of VIP on T-cell differentiation and showed that VIP inhibits Th1 differentiation by interfering directly with the IL-12 Jak2/STAT-4 signalling pathway in T cells [88]. VPAC2 receptor-deficient mice developed exacerbated experimental autoimmune encephalomyelitis with increased Th1/Th17 and reduced Th2/Treg responses [89]. VIP induced Tregs responses and regulated Tregs/Th17 balance [90-92]. As mentioned above, the chronic immune response seen in PD along with the availability of VIP could be protective through induction of a Tregs response. In this context, Reynolds *et al.* reported that Tregs attenuated Th17 cell-mediated nigrostriatal dopaminergic neurodegeneration in a model of Parkinson's disease [83].

4.3. Alpha-Synuclein and VIP

To our knowledge, the normal physiological function of α -synuclein plays a role in the compartmentalization, storage and recycling of neurotransmitters [93]. It has also been suggested that α -synuclein may be associated with the physiological regulation of certain enzymes and increase the number of dopamine transporter molecules [93]. Neurotransmitter release [95] and collaboration with the synaptic SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptors) complex are partially mediated by α -synuclein's role as a molecular chaperone [94]. Alpha-synuclein exists in various conformations in an active equilibrium, is modulated by many factors, comprises internal and external factors that either accelerate or inhibit fibrillation, and degrades by the ubiquitin-proteasome system (UPS) and the autophagy-lysosomal pathway. An *in vivo* study provided evidence that normal soluble α -synuclein is degraded largely by the UPS; however, more complex conformations, including aggregates, are degraded by the autophagy-lysosomal pathway [96]. The finding that α -synuclein in both the monomeric and oligomeric states can be detected in human plasma, cerebrospinal fluid, and other peripheral tissues [97, 98] suggested the idea that α -synuclein is secreted. Although the mechanism of α -synuclein release is not fully known, it appears to be released by exosomes in a calcium-dependent manner and be further degraded after lysosomal inhibition [99].

As mentioned above, inflammation is an important area of research in PD pathogenesis. Glial cells play an important role in the mechanisms of neuroinflammation, and direct transfer of α -synuclein from neurons to astrocytes *in vivo* in transgenic mice overexpressing the human α -synuclein has been demonstrated [100]. In the same study, the authors also found that the secretion of α -synuclein by neurons induced toxicity not only inside the cytoplasm of neighbouring cells but also in the extracellular space [100]. Thus, these results clarify how glial cells are activated, induce chronic inflammation and contribute to the progression of pathology in the brain (Fig. 1).

Alpha-synuclein may contribute to PD pathogenesis in several ways, but it is commonly thought that its abnormal soluble oligomeric conformations, called protofibrils, are the toxic species that mediate deterioration of cellular homeostasis and neuronal death, through effects on several intracellular targets, including synaptic function. In addition, secreted α -synuclein may exert harmful effects on adjacent cells, including seeding of aggregation, thus possibly contributing to disease spread [101]. The relevance of α -synuclein deposition in the pathogenesis of PD is sustained by the fact that the staging of clinical progression of the disease has been correlated with the spreading of α -synuclein pathology in the brain [102]. Alpha-synuclein turned out to be strongly expressed within LBs, particularly in the "halo" of the inclusions. In the PD brain, LBs are mostly found in sites of neuronal loss, that is, the SNc and locus coeruleus; therefore, they were hypothesized to play a pathogenic role in neuronal degeneration. The spread of the pathology to the brain usually takes place with a certain hierarchy. In the initial phases, LBs affect the dorsal motor nuclei and the reticular zone; then, the pathology grows to the medulla oblongata, pontine tegmentum, raphe nuclei, locus coeruleus, and reticular nucleus and later influences the SNc. This stage is thought to coincide with the onset of motor symptoms. In advanced phases, α -synuclein deposits can be found in other brain regions.

As the main element of the LBs and Lewy neurites, α -synuclein is the product of the first gene recognized as associated with PD—SNCA was reported in 1997 by Polymeropoulos *et al.* [103]. Mutations in SNCA (duplications, triplications, or point mutation) cause autosomal dominant forms of PD and are the origin of the risk of developing sporadic PD [104]. Studies [105, 106] suggested that the misfolding of α -synuclein caused it to aggregate and spread in certain sites, where the inflammation induced by it is intimately involved in the pathogenetic dysfunction underlying PD.

Another subject that needs to be investigated is the possible effects of VIP on α -synuclein pathology. There is not yet a direct study showing the effects of VIP on α -synuclein pathology. However, it can be indirectly linked to the findings at hand. Namely, ADNP is a neuron/glial-derived protein that contains a femtomolar active neuroprotective peptide, NAPVSIPQ (NAP) [107]. VIP induced a two- to threefold increase in ADNP mRNA in astrocytes, suggesting that ADNP is a VIP-responsive gene [108]. It was suggested that ADNP could be part of the VIP protection pathway through the femtomolar acting NAP and through putative interactions with other macromolecules. ADNP acts as a chaperon molecule and induces hsp60 [107]. Thus, it may have a positive effect on α -synuclein pathology by acting as a chaperone molecule via ADNP. Of course, the correctness of this suggestion requires additional investigation.

5. BACKGROUND OF VIP AND PARKINSON'S DISEASE MODELS

So far, we have focused on the direct and indirect effects of the VIP on possible neurodegeneration mechanisms, including Parkinson's disease. There are also a number of important studies of the effects of VIP on Parkinson's disease pathology in the disease models that we have mentioned above. Let us look at these studies in more detail.

In a study by Offen *et al.*, while low concentrations of VIP protected against dopamine and 6-OHDA toxicity in human and rat neuronal cells culture, VIP did not rescue neurons from death associated with MPTP administration [50]. Because dopamine toxicity is linked to the redox state of cellular glutathione, they examined neuroprotection in cells depleted of reduced GSH. Therefore, in the same study, when they applied VIP with buthionine sulfoximine (BSO), which is a selective GSH inhibitor, VIP provided neuroprotection against BSO toxicity [50]. In a study conducted by Delgado and Ganea in the MPTP-induced Parkinson mouse model, activated microglial cells play an active role in the pathology of this model, and intracerebral VIP administration meaningfully decreased MPTP-induced neuronal loss in basal ganglia [109]. The same investigators showed that this neuroprotective effect was achieved by suppressing microglial activation and cytotoxic mediators such as iNOS, IL-1 β and TNF- α . Similarly, protection against neurons has been achieved with regulation of microglial activation and reduction of astrogliosis in the MPTP mouse model with a VPAC2 receptor agonist [110].

Intracranial administration of 6-OHDA to rodent striatum is one of the valuable Parkinson models. In this model, the neurotoxin 6-OHDA has two mechanisms of action related to dopaminergic neurons: it immediately creates free radicals and it is a strong inhibitor of the mitochondrial respiratory chain complexes I and IV of the neuron [111]. Thus, the events leading to neurodegeneration are initiated. At this point, it is worth mentioning a series of complementary studies that have been conducted by us exploring the effects of VIP in the 6-OHDA model of PD (they are summarized in Fig. 2). Unilaterally generated 6-OHDA Parkinson model rats were systemically treated with VIP to investigate effects on apomorphine-induced rotational behaviour, extracellular dopamine levels and striatal histology [112]. As a result, VIP has reversed the impaired rotational deficits, although it could not recover the diminished levels of striatal dopamine. On the other hand, neuronal losses and demyelination in the striatum were reduced by VIP treatment. It was observed that the electron microscopic appearance of mast cells differed between VIP-treated and non-treated groups, suggesting that the therapeutic effect of VIP may be mediated by brain mast cells [112]. In the following experiment, we measured gamma-aminobutyric acid (GABA) levels in the ventral-anterior thalamus (VATh), the outlet of the basal ganglion circuit, and found that GABA levels were significantly increased in VATh [113]. Although striatal dopamine levels did not change after VIP treatment,

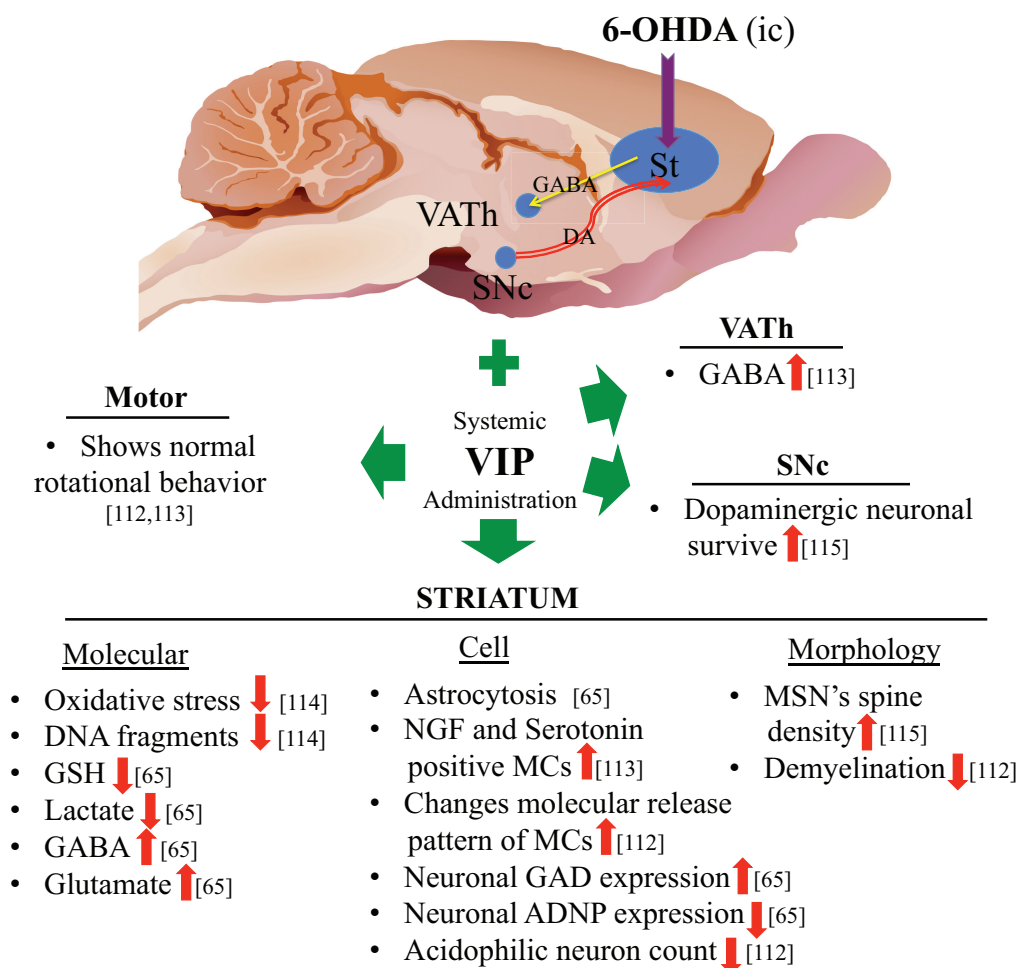


Fig. (2). The effects of systemically administered (25 ng/kg) VIP in the 6-OHDA Parkinsonian rat model (DA: dopamine, GABA: gamma aminobutyric acid, St: striatum, VATH: ventral-anterior thalamus, SNc: substantia nigra compacta, MSNs: medium spiny neurons, GSH: glutathione, NGF: neuronal growth factor, MCs: mast cells, GAD: glutamic acid decarboxylase, ADNP: activity-dependent neuroprotective protein).

the improvement in motor functions were attributed to increased GABA levels in VATH. In the same study, VIP treatment also significantly increased the number of serotonin and NGF-positive mast cells in the striatum [113]. This finding, along with the data from previous studies, provided insight that mast cells could mediate the neuroprotective effects of VIP. Mast cells, as another important type of immune cell in the brain, can be considered to take part in both neurodegeneration and neuroprotection processes of the brain. Our following study showed that VIP had antioxidant and anti-apoptotic activity against 6-OHDA toxicity in the rat striatum [114]. Increased oxidative stress, lipid peroxidation and DNA fragments in the striatum after 6-OHDA injection were significantly reduced by VIP treatment [114]. Another important finding of this study was that although the 6-OHDA administration did not change the striatal NO levels, VIP treatment significantly reduced NO levels in the striatum [114]. These findings could strongly support the suggestion that VIP is using another anti-inflammatory pathway for possible protection. Striatal medium spiny neurons (MSNs) are the main input neurons that collect all the information coming from SNc, cerebral cortex and the thalamus, and they participate in the regulation of direct and indirect pathways in the basal ganglion circuit. Our subsequent study, using the same Parkinson model, showed that VIP increases the spine density of MSNs in the striatum [115]. VIP also increased the total number of tyrosine hydroxylase positive neurons, a sign of dopaminergic activity, in the SNc

[115]. It can be concluded that VIP was possibly involved in synaptic plasticity in the striatum. Our latest study showed that 6-OHDA lesions significantly increased the density of astrocytes in the striatum. 6-OHDA-induced astrogliosis was significantly suppressed by VIP treatment [65]. VIP treatment also reduced GSH level but enhanced GABA and glutamate levels in the striatum.

Striatal MSNs are mostly GABAergic, and the astrocyte-neuron-lactate shuttle hypothesis suggests that during neuronal activity, astrocytes respond to glutamatergic activation by lactate release. Extracellular astrocyte-derived lactate wheels the firing rate and excitability of the neurons, particularly GABAergic neurons. VIP treatment also increased intra-striatal GABA and glutamate concentrations, while lactate and antioxidant GSH levels were reduced [65]. Finally, it was seen in the same study, VIP induced an increase in the expression of GAD and ADNP in neurons rather than astrocytes [65]. It seems that activated astrocytes may constitute the first line of defence for neuronal protection against 6-OHDA toxicity. VIP seems to be involved in neuroprotection either indirectly or directly, by suppressing astrogliosis or inducing GABA and ADNP release from neurons, respectively. In conclusion, several mechanisms that may be associated with the therapeutic effects of VIP on the pathology of Parkinson's disease have been explored by complementary studies in the same animal model.

5.1. What does VIP hold for the future treatment of Parkinson's disease?

For the future treatment of Parkinson's disease, the following are some of the relevant actions:

- i) Has antioxidant and anti-inflammatory action,
- ii) Has modulatory effects on the activity of mast cells, microglial cells and astrocytes,
- iii) Inhibits microglial (M_1) pro-inflammatory activity, and induces microglial (M_2) anti-inflammatory responses,
- iv) Induces Tregs response,
- v) Induces ADNP expression.

We strongly suggest that VIP is holding two additional important advantages for PD treatment.

- 1) Although it needs to be elucidated, our results provide an insight that VIP-induced elevation of GABA and glutamate can trigger neuronal repair by neural stem/progenitor cells (NSPCs) in adult brain.
- 2) ADNP could be a good candidate to reduce α -synuclein misfolding aggregation.

GABA is a major inhibitory neurotransmitter in the adult brain, has emerged as a key player in regulating multiple steps of adult neurogenesis. GABA regulates the development of NSPCs and their neuronal progeny in the adult brain. GABA also plays a potential role as a sensor of neuronal activity and key regulator of the speed/extent of adult mammalian neurogenesis.

Finally, VIP holds two cards against Parkinson's pathology for the future: GABA for neurogenesis and ADNP against α -synuclein aggregation.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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